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IL-4 IS REQUIRED FOR THE IgE AND IgG1 INCREASE AND IgG1 AUTOANTIBODY FORMATION IN MICE TREATED WITH MERCURIC CHLORIDE[1]

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Previous studies have established that in susceptible mouse strains, such as A.SW (H-2^s), repeated injections of subtoxic doses of $HgCl_2$ induce increased serum levels of total IgE and IgG1, high serum titers of antinucleolar autoantibodies (ANolA), and immune-complex glomerulonephritis. Moreover, it has been shown that susceptibility is determined by H-2A^s and that Th cells are required for the induction of these immunopathologic alterations by $HgCl_2$. In the present study we showed that treatment in vivo with anti-IL-4 mAb completely abrogated the $HgCl_2$-induced increase in total IgE and partially inhibited the increase in IgG1, but failed to suppress the increase in IgG2A. Furthermore, we showed that IL-4 influences the pattern of IgG subclass distribution among ANolA of $HgCl_2$-treated mice. Whereas treatment with anti-IL-4 mAb significantly reduced the titers of IgG1 ANolA, it increased those of IgG2A, IgG2B, and IgG3 ANolA. Thus, these results show that IL-4 contributes to the optimal formation in vivo of murine IgG1 and that it is involved in the autoantibody formation of a systemic autoimmune disease. The available evidence suggests that $HgCl_2$ induces an increased production of IL-4 by Th2 cells. If this is correct, it implies that MHC class II alleles determine whether the preferential response to $HgCl_2$ is made by Th1 or Th2 cells and, hence, the type of immunopathologic alterations ensuing.

Upon continual administration of subtoxic doses of $HgCl_2$, susceptible mouse strains exhibit increased serum levels of IgG, especially IgG1, and IgE, very high titers of ANolA,[3] and immune-complex glomerulonephritis (1–4). $HgCl_2$-induced ANolA primarily react with fibrillarin, a protein associated with the small nucleolar RNA U3 (5, 6). The same type of autoantibody occurs in human idiopathic scleroderma (5, 6).

Susceptibility to the pathologic alterations inducible by $HgCl_2$ is determined by both MHC class II loci and unknown non-MHC loci. Highly susceptible mouse strains, such as A.SW and B10.S, all carry the H-2^s haplotype, whereas strains DBA/2 and B10.D2 (both H-2^d) are resistant (2–4). Within H-2, A^s codes for the extreme susceptibility to $HgCl_2$-induced ANolA formation and the other pathologic alterations mentioned above whereas A^d codes for resistance (3; C. Pfeiffer, L. Velthuysen, and J. A. Bruijn, unpublished observations).

In both rats and mice, induction of lymphadenopathy, increased IgE and IgG serum levels, and autoimmune disease by $HgCl_2$ were shown to require, respectively, T and Th cells (5, 7–10). Moreover, the IgE increase and autoantibody formation inducible by $HgCl_2$ can be transferred to untreated animals by CD4$^+$ T cells obtained from $HgCl_2$-treated syngeneic donors (11). However, the mechanism by which $HgCl_2$ activates Th cells is not understood, and the same is true for the mode of cooperation between $HgCl_2$-activated Th cells and B cells. One possibility is that Th cells activated by $HgCl_2$ follow their intrinsic program and secrete interleukins, such as IL-4 (12, 13). IL-4, in turn, would enable B cells to switch to the production of IgG1 and IgE antibodies and enhance their production (14–17). If this assumption was correct, the $HgCl_2$-induced increase in total serum IgE and IgG1 as well as the formation of IgG1 autoantibodies should depend on IL-4 and, hence, be inhibited by administration in vivo of monoclonal antibody to IL-4. In the present investigation we show that this is the case.

MATERIALS AND METHODS

Mice. Specific pathogen-free female A.SW mice were purchased from Harlan Olac Ltd. (Bicester, Oxon, UK). Female BALB/c nu/nu mice were obtained from the Zentralinstitut für Versuchstierkunde (Hannover, FRG). All animals were 6 to 8 wk old at the onset of the experiments.

Treatment with $HgCl_2$. $HgCl_2$ (analytical grade, Merck, Darmstadt, FRG) was prepared in 0.9% sterile, pyrogen-free saline. Mice received injections of 0.5 mg of $HgCl_2$ per kg of body weight s.c. three times per week throughout the entire duration of the experiments (3, 4, 6). Control animals received saline only. Mouse serum was obtained by bleeding from the retro-orbital plexus, performed under CO_2 anesthesia.

Immunological reagents. Cells from the hybridoma 11B11, which secrete rat IgG1 mAb against mouse IL-4 (18), were generously provided by Dr. W. E. Paul (Laboratory of Immunology, The National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD). As a control mAb we used rat IgG1 from the hybridoma LO-DNP-2, which is directed against DNP; this hybridoma was a gift from H. Bazin (Medical Faculty of the University of Louvain, Brussels, Belgium). Ascites from both hybridomas was obtained by injecting 10^6 hybridoma cells i.p. into pristane-treated BALB/c nu/nu mice. The resulting ascites was centrifuged (400 × *g*, 20 min), sterile-filtered, and stored in aliquots at −70°C. Concentrations of rat IgG in the ascitic fluid were measured by a sandwich

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[3] Abbreviations used in this paper: ANolA, antinucleolar autoantibodies.

ELISA (4) specific for rat IgG using affinity-purified goat $F(ab)_2$ antibodies against rat IgG for coating and affinity-purified, peroxidase-labeled goat anti-rat Fc-γ (Jackson Immuno Research Laboratories, West Grove, PA) as a second antibody. The concentrations of both kinds of ascitic fluid were adjusted to 1 mg of rat IgG/ml using sterile, pyrogen-free saline. The endotoxin concentration of ascitic fluid containing 1 mg/ml of 11B11 mAb was 8.5, and that containing 1 mg/ml of LO-DNP-2 was 6.4 *Escherichia coli* U/ml, as determined by chromogenic assay (19). Sera from three normal mice of our colony were assayed for comparison and found to contain 5.0, 5.5, and 9.4 *E. coli* U/ml.

For use in a sandwich IgE ELISA mAb 95.3 against mouse IgE (20) was purifed as described by M. Baniyash (The Weizmann Institute of Science, Rehovot, Israel) who kindly provided this mAb. One milligram of purified EM 95.3 was biotinylated with 400 μg of biotinyl *N*-hydroxyl succinimide ester (E-Y Laboratories, Inc., San Mateo, CA). For indirect immunofluorescence studies, the following affinity-purified antisera were used: goat-anti-mouse IgA, goat-anti-mouse IgM, rabbit-anti-mouse IgG1, goat-anti-mouse IgG2A, goat-anti-mouse IgG2B, and goat-anti-mouse IgG3; all of these were conjugated with FITC (Nordic Immunological Laboratories, Tilburg, The Netherlands).

Treatment with monoclonal antibodies. MAb to IL-4 and DNP, respectively, were administered i.p. at a dose of 1 mg of mAb per injection. In the first experiment, mice were injected with mAb 1 day before and 7 days after the first injection of $HgCl_2$, respectively, of saline. In the second experiment, a third injection of anti-IL-4 mAb was given on day 21.

ELISA for mouse IgE, IgG1, and IgG2A. Total serum IgE, IgG1, and IgG2A were determined by sandwich ELISA (4) using the following affinity-purified antibodies in the solid face: rabbit anti-mouse IgE (MIAB, Uppsala, Sweden), rabbit anti-mouse L chain antiserum (Bionetics, Charleston, SC) for IgG1, and goat anti-mouse L chain antiserum (Southern Biotechnology Associates, Birmingham, AL) for IgG2A. As a second antibody we used biotinylated mAb EM 95.3 anti-mouse IgE, affinity-purified, biotinylated rabbit-anti-mouse IgG1 (Zymed, San Francisco, CA), and affinity-purified, biotinylated goat anti-mouse IgG2A (Southern Biotechnology Associates), respectively.

Immunofluorescence. ANolA in serially diluted mouse sera were determined by indirect immunofluorescence; the initial serum dilution was 1/10. The highest serum dilution at which nucleolar fluorescence could be seen was called ANolA titer (3).

Statistical analysis. Serum Ig concentrations are shown as means ± SEM. Reciprocal serum titers of ANolA are shown as geometrical means ± SEM. A one-tailed *t*-test was used to test the significance of differences between the various groups of animals.

RESULTS

Serum levels of total IgE and IgG1: effects of two injections of anti-IL-4 mAb. A.SW mice were injected with either $HgCl_2$ or saline, as described under *Materials and Methods.* The effect of this treatment on total serum IgE levels is shown in Figure 1*A*. Whereas there was no IgE increase in the saline-treated group (mean serum levels varying from 0.16 to 0.17 μg of IgE/ml), the $HgCl_2$-treated mice showed a 160-fold increase in IgE at week 2 (27.0 ± 2.5 μg of IgE/ml). After week 2, the IgE levels rapidly declined but were still increased by a factor of 51 at the end of the experiment, i.e., in week 6 when compared with those of saline-treated control mice ($p < 0.001$). To study whether IL-4 was involved in the increased IgE levels, groups of $HgCl_2$-treated and saline-treated mice received two injections of anti-IL-4 mAb, and control groups received two injections of anti-DNP mAb. MAb were administered 1 day before and on day 7 after the first injection of $HgCl_2$ or saline. As can be seen in Figure 1*A*, the serum IgE concentrations of mice treated with both $HgCl_2$ and anti-IL-4 mAb were significantly reduced in the first 3 weeks in comparison with mice treated with $HgCl_2$ alone. Mice treated with both $HgCl_2$ and anti-DNP mAb also showed somewhat reduced levels of serum IgE, but these were not as low as those seen in mice injected with $HgCl_2$ and anti-IL-4. No, or only an insignificant increase in IgE levels was measurable in control mice treated with saline and either anti-IL-4 (mean serum levels ranging from 0.16 to 0.26 μg of IgE/ml) or anti-DNP mAb (mean serum levels ranging from 0.2 to 1.8 μg of IgE/ml).

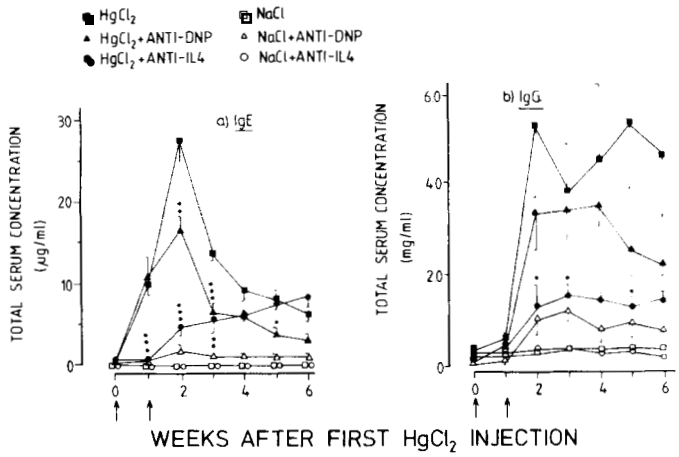

Figure 1. Effects of two injections of anti-IL-4 mAb on the $HgCl_2$-induced increase of IgE (*A*) and IgG1 (*B*) in the serum of A.SW mice. Groups of six mice received repeated s.c. injections of either $HgCl_2$ or saline. One day before and 7 days after the first injection of $HgCl_2$, respectively, of saline mice were injected i.p. with either 1 mg of anti-IL-4 mAb, 1 mg of anti-DNP mAb (*arrows*), or neither of these. Data are shown as means ± SEM. $*p < 0.01$, $**p < 0.05$, and $***p < 0.001$ compared with the $HgCl_2$-treated group.

In the same experimental groups, we also determined the serum concentrations of IgG1. $HgCl_2$-treated A.SW mice, in addition to their increased IgE levels, showed a 9- to 15-fold increase in total IgG1 levels when compared with saline-treated control mice (Fig. 1*B*). IgG1 reached maximal levels in week 2 but, unlike IgE, still showed a 12-fold increase above control values in week 6 after the first injection of $HgCl_2$. Injection of anti-IL-4 mAb on days 1 and 7 significantly decreased the serum levels of IgG1 in $HgCl_2$-treated mice in weeks 2, 3, and 5. Injection of anti-DNP control mAb also decreased the IgG1 levels, but this was not statistically significant ($p > 0.1$).

Serum levels of total IgE, IgG1, and IgG2A: effects of three injections of anti-IL-4 mAb. The experiment shown in Figure 1 indicated that IL-4 is required for the maximal increase in IgE and IgG1 induced by $HgCl_2$. However, the increase in both IgE and IgG1 was only partially inhibited by the two injections of anti-IL-4 mAb administered. Therefore, in addition to the injections of anti-IL-4 mAb on days −1 and 7, in the next experiment a third i.p. injection of 1 mg of anti-IL-4 mAb was given on day 21. As shown in Figure 2*A*, this treatment completely inhibited the IgE increase and thus indicated an absolute requirement of IL-4 for the IgE increase seen in $HgCl_2$-treated mice. In contrast, the increase in the IgG1 levels in the serum of the same animals was only partially inhibited by anti-IL-4 mAb (Fig. 2*B*), and that of IgG2A was not inhibited at all (Fig. 2*C*).

Effect of two injections of anti-IL-4 mAb on ANolA titers. We confirmed the results of previous studies (2, 6) in that $HgCl_2$-treated A.SW mice developed high serum titers of ANolA of the IgG class, whereas saline-treated control mice failed to do so (Fig. 3). To determine the role of IL-4 in this autoantibody formation, we compared the IgG ANolA titers in three different groups of mice. The animals treated with $HgCl_2$ alone showed IgG ANolA titers of about 1:7,000 and 1:20,000 in week 4 and 6, respec-

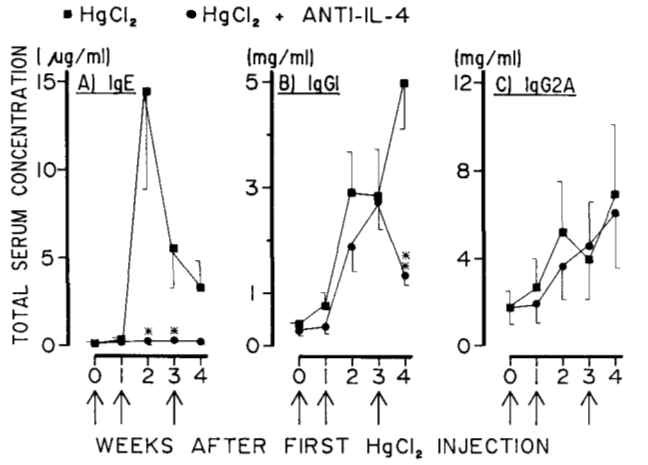

Figure 2. Effects of three injections of anti-IL-4 mAb on the $HgCl_2$-induced increase in IgE (*A*), IgG1 (*B*), and IgG2A (*C*) in the serum of A.SW mice. Two groups of six mice received repeated s.c. injections of $HgCl_2$. One of these groups received an i.p. injection of 1 mg of anti-IL-4 mAb 1 day before and 7 and 21 days after the first $HgCl_2$ injection (*arrows*). Data are shown as means ± SEM. $*p < 0.01$, $**p < 0.05$, and $***p < 0.001$ compared with the $HgCl_2$-treated group.

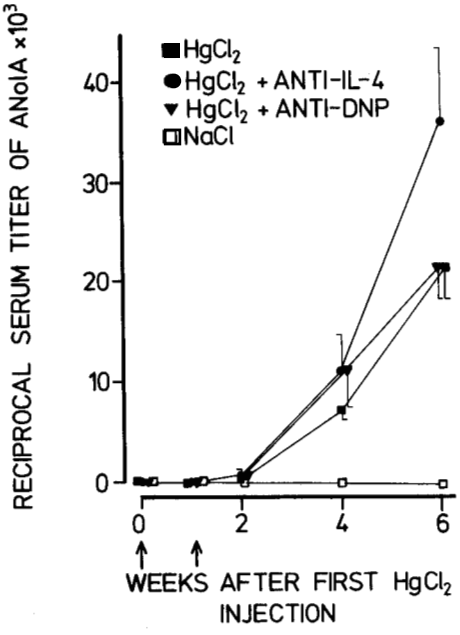

Figure 3. Treatment with anti-IL-4 mAb of $HgCl_2$-injected mice results in a moderate increase of ANolA of the IgG class. Groups of six A.SW mice received repeated s.c. injections of either $HgCl_2$ or saline. One day before and 7 days after the first injection of $HgCl_2$, one group was injected with either 1 mg of anti-IL-4 mAb or 1 mg of anti-DNP mAb (*arrows*). Data are shown as geometric means ± SEM. Differences are not statistically significant ($p > 0.1$).

tively, and very similar titers were found in the group treated with $HgCl_2$ plus anti-DNP mAb. In the group treated with $HgCl_2$ plus anti-IL-4 mAb, however, IgG ANolA titers in week 6 were twofold higher than in the two other groups.

To clarify which IgG subclasses were responsible for this increase of ANolA titers in the mice treated with $HgCl_2$ and anti-IL-4, we analyzed the distribution of ANolA among the various Ig classes and subclasses in mice treated with $HgCl_2$ alone. ANolA of all Ig (sub)classes tested (IgA, IgM, IgG1, IgG2A, IgG2B, IgG3) were detectable. Serum titers of ANolA of all these (sub)classes reached maximal values in week 6 after the first injection of $HgCl_2$ and remained stable until week 15, the end of the observation period (data not shown). Sera obtained from three groups of mice in the 5th wk of $HgCl_2$ treatment were then compared for the IgG subclasses of their ANolA (Fig. 4). The relative contribution to the IgG ANolA of $HgCl_2$-treated mice was IgG1 > IgG2A > IgG2B > IgG3, and essentially the same results were obtained in the control group, which had received $HgCl_2$ plus anti-DNP mAb. However, a completely different pattern of IgG subclass distribution was found in the mice that had been treated with both $HgCl_2$ and anti-IL-4 mAb. Here, a significant reduction of ANolA of the IgG1 subclass was found. Interestingly, this decrease in IgG1 ANolA was accompanied by significant increases of ANolA belonging to the IgG2A, IgG2B, and IgG3 subclasses. Apparently, these increases of non-IgG1 ANolA were so strong that they account for the moderate increase of IgG ANolA in the group treated with $HgCl_2$ and anti-IL-4 mAb, as shown in Figure 3.

DISCUSSION

Our results indicate that in $HgCl_2$-treated H-2^s mice IL-4 is required for the increase in total IgE and, to a lesser extent, the increase in IgG1, but not that in IgG2A. Moreover, IL-4 influences specific autoantibody formation in that it enhances formation of ANolA of the IgG1 subclass and suppresses that of the IgG2A, IgG2B, and IgG3 subclasses.

A 160-fold increase in total serum IgE was detected in $HgCl_2$-treated A.SW mice, and both the magnitude and the kinetics of this increase are very similar to those reported in the rat (21). In the present study, no experiments were undertaken as to the specificity of the IgE

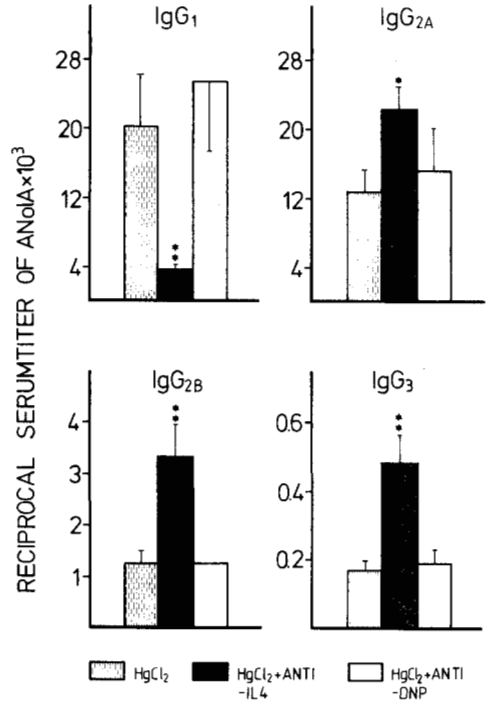

Figure 4. Treatment with anti-IL-4 of $HgCl_2$-injected mice changes the distribution among IgG subclasses of ANolA. Three groups of six A.SW mice received repeated s.c. injections of $HgCl_2$. One day before and 7 days after the first $HgCl_2$ injection, one group was injected with 1 mg of anti-IL-4 mAb and another with 1 mg of anti-DNP mAb. Sera obtained at week 5 of $HgCl_2$ treatment were analyzed. Data are shown as geometric means ± SEM. $*p < 0.01$, $**p < 0.05$, and $***p < 0.001$ compared with the group treated with $HgCl_2$ alone.

antibodies. In rats, the $HgCl_2$-induced IgE increase has been interpreted as being polyclonal in nature, but specific IgE antibodies were also induced if a nominal Ag such as OVA was injected in addition to $HgCl_2$ (21, 22).

The serum concentrations of total IgG1 in the $HgCl_2$-treated A.SW mice showed a 9- to 15-fold increase, similar to what has previously been reported in $HgCl_2$-treated mice (1). Other than in the case of IgE, the IgG1 concentrations of individual $HgCl_2$-treated A.SW mice showed considerable differences, and the kinetics of the IgG1 increase also varied (cf. Figs. 1*B* and 2*B*). Hence, there appears to be a biological variability of IgG1 regulation in the $HgCl_2$ model. Another difference between the kinetics of IgG1 and those of IgE in $HgCl_2$-treated A.SW mice was that the elevation of IgG1 lasted until at least weeks 4 or 6, whereas IgE levels rapidly declined after week 2. Two reasons may account for this difference. First, the biological half-life of murine IgG1 clearly exceeds that of IgE (23). Second, and more importantly, $HgCl_2$-treated H-2^s mice maintain an increased level of splenic B cells containing cytoplasmic IgG1 until at least week 4, whereas an increase in spleen cells containing cytoplasmic IgE is only detectable until week 2 (M. Uhrberg, unpublished observations), which indicates that there is a sustained production of IgG1. Thus, IgE and IgG1 are differently regulated in the $HgCl_2$ model.

A moderately suppressive effect on the $HgCl_2$-induced increase in total IgE and IgG1 was exerted by the mAb to DNP, which served as a specificity control for the anti-IL-4 mAb. Two different reasons, which are not mutually exclusive, might account for this. First, it is conceivable that the slight suppressive effect was due to the endotoxin levels present in the anti-DNP mAb preparation used. It is known that bacterial products, such as endotoxin, induce murine IFN-α (24), and recent findings indicate that IFN-α, like IFN-γ, suppresses murine polyclonal IgE and IgG1 responses in vivo (25). Second, the rat anti-DNP mAb, being a foreign protein, might have induced an immune response that counteracted the production or the effect of the IL-4 induced by $HgCl_2$. On the other hand, however, administration of the anti-DNP mAb failed to suppress the formation of autoantibodies of the IgG1 isotype. Moreover, in the absence of $HgCl_2$, the anti-DNP mAb slightly increased, rather than suppressed, the levels of total IgE and IgG1. Thus, there is no good explanation for the inconsistent effects of the anti-DNP mAb used.

The anti-IL-4 mAb 11B11 produced by Ohara and Paul (18) has proved to be an invaluable tool for assessing the various biologic functions exerted by IL-4 (25–27). Here, we demonstrated that two injections of this anti-IL-4 mAb led to a partial reduction of the IgE increase and that three injections completely abrogated it. These results are consistent with those of Finkelman et al. (25, 26, 28), who found that the increase in total serum IgE (up to 470-fold) induced by *Nippostrongylus brasiliensis* infection of mice could be completely abrogated by the anti-IL-4 mAb 11B11. In contrast to our results, however, they needed a 20-fold higher concentration of anti-IL-4 mAb. One possibility to account for this discrepancy might be that higher IL-4 concentrations are present or that IL-4 is produced over a longer period of time in the parasite model when compared with the $HgCl_2$ model.

Injection of anti-IL-4 mAb into $HgCl_2$-treated mice also led to a significant, albeit incomplete, reduction of the increase in serum IgG1 levels. This finding is consistent with the results of experiments performed in vitro (27, 29–31) as well as those obtained in the murine host-vs-graft model in vivo (32), but differs from the results obtained in *Nippostrongylus*-infected mice, in which anti-IL-4 mAb completely failed to suppress the IgG1 increase (25, 26, 28, 33). The reason for this difference between the in vivo models is unclear. One explanation, mentioned earlier, might be that there is less IL-4 in $HgCl_2$-treated mice than in those infected with parasites, so that it takes less 11B11 mAb to neutralize IL-4 in the former than in the latter.

Reduction of the IgG1 increase in $HgCl_2$-treated A.SW mice was incomplete, irrespective of whether two or three injections of anti-IL-4 were given. This partial suppression of IgG1 in mice that received three injections of anti-IL-4 differs from the complete suppression of IgE observed in the same animals. This confirms the notion (25) that there does not seem to be an absolute requirement of IL-4 for the generation in vivo of polyclonal murine IgG1 responses. On the other hand, however, our data are consistent with those obtained in the host-vs-graft model (32) in that they demonstrate a contribution of IL-4 to the optimal formation in vivo of total murine IgG1. In contrast to the effects on total IgE and IgG1, administration of anti-IL-4 mAb failed to affect the total IgG2A response induced by $HgCl_2$. This is consistent with the lack of effect of IL-4 and anti-IL-4 mAb, respectively, on total IgG2A responses in other experimental systems studied in vivo (25).

The amount of anti-IL-4 mAb used in the present investigation is low compared with that used in other in vivo models (25), and the results obtained with two injections of anti-IL-4 mAb indicated that this treatment schedule fails to neutralize all IL-4. Therefore, with respect to our experiments on the effects of anti-IL-4 treatment on autoantibody formation, we cannot exclude an effect of residual IL-4. With this important reservation in mind, one nevertheless may question whether the process of ANolA formation *per se* does require IL-4. That the basic process might be independent of IL-4 is suggested by the finding that treatment with anti-IL-4 increased the serum titers of IgG, IgG2A, IgG2B, and IgG3 ANolA and decreased only those of IgG1 ANolA. Thus, only the formation of IgG1 ANolA is stimulated by IL-4. This observation made in vivo is consistent with the findings of Snapper et al. (27) made in vitro using LPS blasts. They found that although IL-4 enhances formation of IgG1, it suppresses that of IgG2A, IgG2B, and IgG3. Moreover, our data on autoantibody formation are in remarkable agreement with recent findings made in the host-vs-graft model of systemic autoimmune disease, in which treatment with anti-IL-4 mAb in vivo reduced the formation of anti-ssDNA antibodies of the IgG1, but not of the IgM, IgG3, IgG2A, IgG2B, and IgA isotype (32).

It is likely that $HgCl_2$-treated A.SW mice produce other cytokines in addition to IL-4 and that these also influence the production of IgG subclasses. Thus, stimulation in vitro by $HgCl_2$ of mouse spleen cells induced an increased production of IFN-γ (34). The observed increase of IgG2A ANolA in mice treated with $HgCl_2$ plus anti-IL-4 might be due to small amounts of IFN-γ. IFN-γ has been reported to enhance production of IgG2A while suppressing that

of IgG1, and, at higher concentrations, of IgG2B and IgG3 (35). Conceivably, A.SW mice treated with $HgCl_2$ alone produce some IFN-γ, and this could account for the intermediate titers of IgG2A ANolA in such mice. IL-4 is known to inhibit not only the synthesis but also many of the immunologic effects of IFN-γ (31, 35–37). In A.SW mice treated with $HgCl_2$ plus anti-IL-4, therefore, IFN-γ presumably became more effective, and this may have enhanced the formation of IgG2A ANolA observed in these animals. In contrast to the enhancement of IgG2A ANolA, however, administration of anti-IL-4 mAb failed to further enhance the serum levels of total IgG2A. The reason for this differential effect on specific and total IgG2A is not known.

Our results do not allow us to formally conclude that $HgCl_2$ induced an increased production of IL-4. This is a very likely possibility, however, because recent findings showed increased levels of IL-4 mRNA in $CD4^+$ T cells of $HgCl_2$-treated B10.S ($H\text{-}2^s$), but not B10.D2 ($H\text{-}2^d$) mice (C. Stein and C. Pfeiffer, unpublished observations). Furthermore, $HgCl_2$ treatment of normal murine T cells in vitro induced IL-4.[4] Th cells play a crucial role in the IgE increase and autoimmunity inducible by $HgCl_2$ (5, 7, 8, 10, 11). In the mouse, long-term $CD4^+$ T cell clones can be subdivided into Th1 and Th2 cells on the basis of the different lymphokine profiles they produce. According to this classification, IL-4 is only produced by Th2 cells (38–40). Although it is still a matter of debate whether the Th1/Th2 dichotomy is perhaps an oversimplification and to what extent it reflects the situation in vivo, evidence from two experimental models in vivo clearly indicates that $CD4^+$ T cell responses, made by different inbred mouse strains to the same pathogen or Ag, may result in different lymphokine profiles that correspond to Th1- and Th2-like responses, respectively (41, 42). Moreover, in one of these models, it was shown that whether a given strain preferentially responds by its Th1 or Th2 cells depends on the MHC class II alleles (42). Intriguing parallels to this finding emerge when the Th1/Th2 concept is applied to the $HgCl_2$ model. It is likely that, in $H\text{-}2^s$ mice, $HgCl_2$ preferentially activates the Th2 subset. By contrast, DBA/2 and B10.D2 mice with $H\text{-}2^d$ haplotype fail to produce autoantibodies and increased levels of IgE upon systemic injection of $HgCl_2$ (3, 4). This is not due to a general unresponsiveness to $HgCl_2$ of these two strains, however, because both strains are capable of responding to $HgCl_2$ by a delayed-type hypersensitivity reaction (C. Stringer, unpublished observations), which is a function of Th1 cells (43). Experiments are under way to test how far these MHC-dependent differences in the immunopathologic reactions to the same agent, $HgCl_2$, are due to different cytokine profiles.

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